Research Paper
Impact Factor: 3.567
Peer Reviewed Journal

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

COMPARATIVE STUDY ON BIODEGRADATION OF POLYTHENE (PE) AND CELLULOSE ACETATE PLASTIC (CA) USING NATURAL SOIL FLORA

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Abstract
Polythene is most problematic plastic waste which is an increasing ecological threat; Polythenes are a type of organic polymers having high molecular weight and are commonly derived from different petrochemicals. Polythenes are stable polymers and cannot easily degrade. The current study makes a comparative analysis between the biodegradation of polythene and cellulose acetate plastic using natural soil flora Pseudomonas and Aspergillus niger. The microbes are isolated from Municipal Garbage soil. The polythene and CAP were allowed to degrade in the presence of microbes. Microbes were highly degrading the plastics. In comparison Aspergillus niger is capable of degrading more CAP where Pseudomonas is more potent in polythene degradation. Biodegradation is the natural way to resolve this threat.

Key Words: Biodegradation, Polythene, Cellulose Acetate Plastic (CA), Soil Flora.

Introduction

During the past 25 years, plastic materials have gained widespread use as they have been increasingly used in food, clothing, shelter, transportation, construction and medical industries. The usage and production of plastics is ever increasing due to their various uses. They are lightweight, strong, inexpensive, duration, corrosion resistant and have high thermal and electrical insulation properties. The production of plastic increased from 0.5 million tonnes in 1950 to over 260 million (Thompson et al. 2009) tonnes now. However, most of these conventional plastics are nonbiodegradable or biodegradable at a very slow rate (Sharon et al. 2012). Hence, the accumulation of these materials has been accountable for innumerable environmental hazards like air water and soil pollution and has been a threat to the planet (Hoffmann et al., 2003; Jang et al., 2002, Tokiwa et al. 2009, Sharon et al. 2012).

Degradation of polythene is one of the great challenges for researchers. Biodegradation is the natural way to resolve this hazard. Any physical (like weight loss of sample, tensile strength) or chemical change (like carbon dioxide production) in the material suggests biological degradation by microorganisms. The degradation of a polymer is affected many factors like temperature, moisture, oxygen, sun light, stress, living organisms and contaminants (Shah et al. 2008). Depending on the factors responsible for the degradation of the polymers, polymer degradation methods are categorized into photodegradation, thermo-oxidative degradation and biodegradation. The biodegradation is a natural process of degrading materials through microbes such as bacteria, fungi and algae [Rutkowska et al. 2002]. Many researchers have reported about biodegradation. Oda *et al*. (1998) studied polycaprolactone depolymerase produced by the bacterium *Alcaligenes faecalis*and isolated several bacteria capable of degrading polycaprolactone (PCL) from soil and activated sludge. Webb *et al*. (2000) studied the fungal colonization and biodeterioration of plasticized polyvinyl chloride in *in situ* and *ex situ* conditions and suggested that microbial succession may occur during the long periods of exposure in *in situ* conditions. Microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastic (Gu *et al*., 2000, *Lee et al*., 1991) have reported the biodegradation of degradable plastic polyethylene by phanerochaete and *Streptomyces* species.

The current research is focused on the biodegradation of polythene and cellulose acetate plastics by bacteria and fungi. Among the synthetic plastics waste produced, polythene shares about 64% [Lee et al. 1991]. Plastic polymers are high molecular weight molecules that cannot cross the cell wall. Microorganisms secrete extracellular enzymes (exoenzymes) that can catalyze reactions principally at the boundaries of the plastic polymer. The bio-deterioration seems to be triggered by the formation of a microbial biofilm growing on the surface and inside the plastic. The development of the biofilm is dependent on the composition and the structure of the plastic, but also on the environmental conditions (Lugauskas *et al.*, 2003). Microbial degradation of plastics is caused by enzymatic activities leading to a chain cleavage of the polymer into oligomers and monomers after which they are further metabolized by the microbial cells. Aerobic metabolism results in carbon dioxide and water (Starnecker and Menner, 1996), whereas anaerobic metabolism results in carbon dioxide, water, and methane as the end products, respectively (Gu et al., 2000).

Hence the present study suggests that biodegradation is one of the best eco friendly and cost effective methods.

Materials and Methods

Plastic: Polythene and cellulose acetate plastic (CA) were collected from the local market shops.

Sample Collection for Polythene Degrading Microbes

Dry soil sample present on the naturally buried polythene bags was collected from domestic waste disposal site dumped with household garbage of Devinagar, Bellary, Karnataka, India, to isolate polythene degrading microbes.

Fig. 1: Source of Garbage Soil

Isolation and Characterization of Microorganisms

1% (w/v) soil sample solution was prepared and serially diluted. For each dilution triplicate Luria bertani (LB) agar plates were made to isolate bacteria and potato dextrose agar (PDA) plates to isolate the fungi. The cultured plates were incubated at room temperature for 2-3 days. The developed colonies were isolated and sub-cultured repeatedly to get the pure culture and preserved as slants at 4ºC.

From the isolated cultures, one bacteria and one fungus were successfully screened for plastic biodegradation property. The bacteria were further identified on the basis of colony characterization, grams staining and biochemical analysis (IMVIC) according to Bergey's manual (Holt et al. 1994). Fungi identification was done by lacto phenol cotton blue staining test following the keys Raper and Fennell (Raper et al. 1987).

Biodegradation of Cellulose Acetate (CA) Plastic

CA-GPY medium (Peptone, Yeast Extract, Glucose, Tripotassium hypophosphate, Magnesium sulphate and Cellulose Acetate) of 100ml was prepared and sterilized in two different 250ml conical flasks except Cellulose Acetate. 0.1gm of CA was weighed and sterilized with disinfectants and added to the sterilized CA-GPY medium then, inoculated with loop full of bacterial and fungal cultures separately and incubated on mechanical shaker at room temperature. The degradation period was maintained for 30days and the weight loss was measured at 10days of interval.

Fig. 2: Method of Plastic Supplement to Microbial Culture and Incubation

Biodegradation of Polythene (PE) Plastic

PE-GPY medium (Peptone, Yeast Extract, Glucose, Tripotassium hypophosphate, Magnesium sulphate and Cellulose Acetate) of 100ml was prepared and sterilized in two different 250ml conical flasks except Cellulose Acetate. 0.1gm of PE was weighed and sterilized with disinfectants and added to the sterilized CA-GPY medium then, inoculated with loop full of bacterial and fungal cultures separately and incubated on mechanical shaker at room temperature. The degradation period was maintained for 30days and the weight loss was measured at 10days of interval.

Results and Discussion

Biological degradability of polymers by microorganisms decreases with increase in the molecular weight of the polymer. With increase in molecular weight, there is decrease in polymer solubility which makes it unfavorable for microbial attack as the polymer needs to be assimilated into the bacterial cell membrane and broken down by cellular enzymes. Repeating units

of polymers like monomers, dimers and oligomers are easily degraded and mineralized (Shah et al., 2008). Biodegradation is enhanced by abiotic hydrolysis, photo-oxidation and physical disintegration. These processes enhance the surface area of the polymer and reduce its molecular weight; facilitating microbial degradation (Singh et al. 2007).

The biodegradation involves microbial agents and does not require heat. Organic material can be degraded in two ways either aerobically or anaerobically. In landfills and sediments, plastics are degraded anaerobically while in composite and soil, aerobic biodegradation takes place. Aerobic biodegradation leads to the production of water and CO_2 and anaerobic biodegradation results in the formation of water, CO_2 and methane as end products [Revie 2000]. Since plastic polymers such as PE and PS are hydrophobic, forming a stable biofilm requires that the bacterial surface will also be hydrophobic.

Identification of Plastic Degrading Microorganisms

The *Pseudomonas* spp. was isolated from household garbage contaminated area and characterized basing on their morphological and biochemical characteristics and identified the bacteria using Bergey's manual. Lacto-phenol cotton blue staining and following the keys of Raper and Fennell identified the screened fungus as *Aspergillus niger* (Raper et al. 1987), which were listed in table.1.

Table 1: Morphological and Biochemical Characteristics of Pseudomonas Spp. and Aspergillus Niger Isolated from Household Garbage Contaminated

Characteristics	Pseudomonas
Color	Bluish green
Gram staining	-
Motility	+
Indole	-
Methyl red	-
Voges Proskauer	-
Citrate	+
Urease	-
Nitrate	+
Triple sugar iron	+
Hydrogen sulphide	+
Catalase	+
Denitrification	+
Gelatin liquification	+
Microoganism - Fungi	**Aspergillus niger**
Differential Media	Potato Dextrose Agar (PDA)
Lacto Phenol Cotton Blue Test	+ve
Morphology	Brown to Black spores

Analysis of Cellulose Acetate (CA) and Polythene (PE) Degradation Using Pure Microorganisms

Earlier studies on soil microorganisms reveal their active association in biodegradation of plastic films and polyethylene bags (Kathiresan.2003). 0.11 gm of CA was taken as initial weight, after 30days of incubation the weight of CA was reduced to 0.09gm by bacteria where the fungi degraded it to 0.02gm which is represented in Table.2.

Table 2: Weight Loss Analysis for Cellulose Acetate (CA) Using the Pseudomonas Spp. and Aspergillus Niger

S. No	Organism	Initial weight (gm)	Inc. time	Final weight (gm)	Difference in weight (gm)	Percentage (%)
1	Bacteria	0.11	30 days	0.09	0.02	18.18
2	Fungi	0.11	30 days	0.02	0.09	81.00

Table 3: Weight Loss Analysis for Polythene (PE) Using the Pseudomonas Spp. and Aspergillus Niger

S. No	Organism	Initial Weight (gm)	Inc. Time	Final Weight (gm)	Difference in Weight (gm)	Percentage (%)
1	Bacteria	0.23	30 days	0.17	0.06	26.00
2	Fungi	0.23	30 days	0.18	0.05	23.33

Table.3 shows that the initial weight of PE, which is 0.23gm, was reduced to 0.17gm and 0.18gm by bacteria and fungi respectively. From the results it is clearly tells that *Pseudomonas* sp. (bacteria) is showing better degradation of PE where *Aspergillus niger* (fungi) is acing good on CA than PE.

Microbial degradation of CA and PE (plastics) are mainly due to catalytic action of enzymes as well as their growth conditions such as, pH, temperature, moisture content, oxygen, nutrients, etc., Cellulase and amylase enzymes which were produced by *Pseudomonas* sp. and *Aspergillus niger* played major role in degradation of plastics that cleave polymeric plastics into oligomers, dimers or monomers.

Conclusion

Recycling of plastics is not always efficiently possible so it is necessary to find diverse methods of biodegradation of plastics. The current research indicates that *Aspergillus niger* (fungi) is capable of degrading more CA compared to *Pseudomonas* sp. (bacteria) but in case of PE *Pseudomonas* sp. Was found to be more potent enough when compared to *Aspergillus niger.*

Biodegradation is one of the best, low cost, efficient and eco-friendly. Degradation treatments capable of reducing and even eliminating plastics are of great environmental interest. Among biological agents, microbial enzymes are one of the most powerful tools for the biodegradation of plastics. There is a huge demand in exploring these microbes which can grow in different conditions and, under specific stress conditions, may be directed to grow and use the plastic carbon polymers as energy source.

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